

WHAT IS CLAIMED IS:

1. A nucleic acid-lipid particle composition for introducing a nucleic acid
2 into a cell, said particle comprising: a cationic lipid, a conjugated lipid that inhibits
3 aggregation of particles, a nucleic acid and an endosomal membrane destabilizer.
1. 2. The nucleic acid-lipid particle composition of claim 1, wherein said
2 endosomal membrane destabilizer is outside said nucleic acid-lipid particle.
1. 2. 3. The nucleic acid-lipid particle composition of claim 1, wherein said
2 endosomal membrane destabilizer is both outside and inside said nucleic acid-lipid particle.
1. 2. 4. The nucleic acid-lipid particle composition of claim 1, wherein said
2 endosomal membrane destabilizer is Ca^{++} ion.
1. 2. 5. The nucleic acid-lipid particle composition of claim 4, wherein the
concentration of Ca^{++} ion is from about 0.1 mM to about 100 mM.
1. 2. 6. The nucleic acid-lipid particle composition of claim 5, wherein the
concentration of Ca^{++} ion is from about 1 mM to about 20 mM.
1. 2. 7. The nucleic acid-lipid particle composition of claim 1, wherein said
2 particle has a median diameter of less than about 150 nm.
1. 2. 3. 4. 5. 6. 8. The nucleic acid-lipid particle composition of claim 1, wherein said
cationic lipid is a member selected from the group consisting of N,N-dioleyl-N,N-
dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide
(DDAB), N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-
(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), and N,N-
dimethyl-2,3-dioleyloxy)propylamine (DODMA), and combinations thereof.
1. 2. 9. The nucleic acid-lipid particle composition of claim 1, wherein said
particle further comprises an additional noncationic lipid.
1. 2. 10. The nucleic acid-lipid particle composition of claim 9, wherein said
noncationic lipid is selected from the group consisting of DOPE, POPC, and EPC.

1 **11.** The nucleic acid-lipid particle composition of claim 1, wherein said
2 particle comprises a functional group that facilitates Ca⁺⁺ ion chelation.

1 **12.** The nucleic acid-lipid particle composition of claim 1, wherein said
2 conjugated lipid that inhibits aggregation of particles has the formula



I

3
4 wherein: A is a lipid moiety;
5 W is a hydrophilic polymer; and
6 Y is a polycationic moiety.

1 **13.** The nucleic acid-lipid particle composition of claim 12, wherein W is a
2 polymer selected from the group consisting of PEG, polyamide, polylactic acid, polyglycolic
3 acid, polylactic acid/polyglycolic acid copolymers and combinations thereof, said polymer
4 having a molecular weight of about 250 to about 7000 daltons.

1 **14.** The nucleic acid-lipid particle composition of claim 12, wherein Y has
2 at least 4 positive charges at a selected pH.

1 **15.** The nucleic acid-lipid particle composition of claim 12, wherein Y is a
2 member selected from the group consisting of lysine, arginine, asparagine, glutamine,
3 derivatives thereof and combinations thereof.

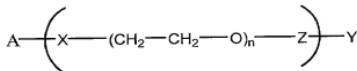
1 **16.** The nucleic acid-lipid particle composition of claim 12, wherein A is a
2 member selected from the group consisting of a diacylglycerolyl moiety, a dialkylglycerolyl
3 moiety, a N-N-dialkylamino moiety, a 1,2-diacyloxy-3-aminopropane moiety and a 1,2-
4 dialkyl-3-aminopropane moiety.

1 **17.** The nucleic acid-lipid particle composition of claim 12, wherein W is
2 PEG.

1 **18.** The nucleic acid-lipid particle composition of claim 12, wherein W is a
2 polyamide polymer.

1 **19.** The nucleic acid-lipid particle composition of claim 12, wherein W has
2 a molecular weight of about 250 to about 2000 daltons.

1 **20.** The nucleic acid-lipid particle composition of claim 17, having the
2 general structure of Formula II:



II

3 wherein

4 X is a member selected from the group consisting of a single bond or a
5 functional group covalently attaching said lipid to at least one ethylene oxide unit;

6 Z is a member selected from the group consisting of a single bond or a
7 functional group covalently attaching said at least one ethylene oxide unit to a cationic group;
8 and

9 n is an integer having a value of between about 6 to about 50.

10 **21.** The nucleic acid-lipid particle composition of claim 20, wherein

1 X is a member selected from the group consisting of a single bond,
2 phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,
3 phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate,
4 amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

5 **22.** The nucleic acid-lipid particle composition of claim 20, wherein

1 Z is a member selected from the group consisting of a single bond,
2 phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,
3 phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate,
4 amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

5 **23.** The nucleic acid-lipid particle composition of claim 20, wherein

6 A is a diacylglycerolyl moiety;
1 X is phosphoethanolamido;
2 Z is NR, wherein R is a hydrogen atom; and
3 Y is a member selected from the group consisting of about 1 to about 10 basic
4 amino acids or derivatives thereof.

1 **24.** The nucleic acid-lipid particle composition of claim 23, wherein

2 A is a diacylglycerol moiety having 2 fatty acyl chains, wherein each acyl
3 chain is independently between 2 and 30 carbons in length and is either saturated or has
4 varying degrees of saturation.

1 **25.** The nucleic acid-lipid particle composition of claim **23**, wherein
2 Y is a member selected from the group consisting of lysine, arginine,
3 asparagine, glutamine, derivatives thereof and combinations thereof.

1 **26.** The nucleic acid-lipid particle composition of claim **23**, wherein
2 A is a diacylglycerol moiety having 2 fatty acyl chains, wherein each acyl
3 chain is a saturated C-18 carbon chain; and
4 Y is a cationic group having 4 lysine residues or derivatives thereof.

1 **27.** The nucleic acid-lipid particle composition of claim **1**, wherein said
2 conjugated lipid that inhibits aggregation of particles is a PEG-lipid.

1 **28.** The nucleic acid-lipid particle composition of claim **27**, wherein said
2 PEG-lipid is PEG-ceramide.

1 **29.** The nucleic acid-lipid particle composition of claim **28**, wherein the
2 ceramide of said PEG-ceramide comprises a fatty acid group having about 8 to about 20
3 carbon atoms.

1 **30.** The nucleic acid-lipid particle composition of claim **28**, wherein said
2 PEG-lipid is PEG-phosphatidylethanolamine.

1 **31.** The nucleic acid-lipid particle composition of claim **1**, wherein said
2 conjugated lipid that inhibits aggregation of particles is an ATTA-lipid.

1 **32.** The nucleic acid-lipid particle composition of claim **1**, wherein said
2 nucleic acid is selected from the group consisting of a plasmid, an antisense oligonucleotide,
3 and a ribozyme.

1 **33.** A method of introducing a nucleic acid into a cell, said method
2 comprising:
3 contacting said cell with a nucleic acid-lipid particle composition, said particle
4 comprising a cationic lipid, a conjugated lipid that inhibits aggregation of particles, and a
5 nucleic acid; and an endosomal membrane destabilizer.

1 **34.** The method of introducing a nucleic acid into a cell of claim **33**,
2 wherein said endosomal membrane destabilizer is outside said nucleic acid-lipid particle.

1 **35.** The method of introducing a nucleic acid into a cell of claim **33**,
2 wherein said endosomal membrane destabilizer is Ca^{++} ion.

1 **36.** The method of introducing a nucleic acid into a cell of claim **35**,
2 wherein the concentration of Ca^{++} ion is from about 0.1 mM to about 100 mM.

1 **37.** The method of introducing a nucleic acid into a cell of claim **36**,
2 wherein the concentration of Ca^{++} ion is from about 1 mM to about 20 mM.

1 **38.** The method of introducing a nucleic acid into a cell of claim **33**,
2 wherein said particle has a median diameter of less than about 150 nm.

1 **39.** The method of introducing a nucleic acid into a cell of claim **33**,
2 wherein said cationic lipid is a member selected from the group consisting of N,N-dioleyl
3 N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium
4 bromide (DDAB), N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride
5 (DOTAP), N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA),
6 and N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), and combinations thereof.

1 **40.** The method of introducing a nucleic acid into a cell of claim **33**,
2 wherein said particle further comprises an additional noncationic lipid.

1 **41.** The method of introducing a nucleic acid into a cell of claim **40**,
2 wherein said noncationic lipid is selected from the group consisting of DOPE, POPC, and
3 EPC.

1 **42.** The method of introducing a nucleic acid into a cell of claim **33**,
2 wherein said particle comprises a functional group that facilitates Ca^{++} ion chelation.

1 **43.** The method of introducing a nucleic acid into a cell of claim **33**,
2 wherein said conjugated lipid that inhibits aggregation of particles has the formula



4 wherein: A is a lipid moiety;
5 W is a hydrophilic polymer; and
6 Y is a polycationic moiety.

1 **44.** The method of introducing a nucleic acid into a cell of claim **43**,
2 wherein W is a polymer selected from the group consisting of PEG, polyamide, polylactic
3 acid, polyglycolic acid, polylactic acid/polyglycolic acid copolymers and combinations
4 thereof, said polymer having a molecular weight of about 250 to about 7000 daltons.

1 **45.** The method of introducing a nucleic acid into a cell of claim **43**,
2 wherein Y has at least 4 positive charges at a selected pH.

1 **46.** The method of introducing a nucleic acid into a cell of claim **43**,
2 wherein Y is a member selected from the group consisting of lysine, arginine, asparagine,
3 glutamine, derivatives thereof and combinations thereof.

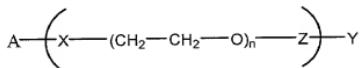
1 **47.** The method of introducing a nucleic acid into a cell of claim **43**,
2 wherein A is a member selected from the group consisting of a diacylglycerolyl moiety, a
3 dialkylglycerolyl moiety, a N-N-dialkylamino moiety, a 1,2-diacyloxy-3-aminopropane
4 moiety and a 1,2-dialkyl-3-aminopropane moiety.

1 **48.** The method of introducing a nucleic acid into a cell of claim **43**,
2 wherein W is PEG.

1 **49.** The method of introducing a nucleic acid into a cell of claim **43**,
2 wherein W is a polyamide polymer.

1 **50.** The method of introducing a nucleic acid into a cell of claim **43**,
2 wherein W has a molecular weight of about 250 to about 2000 daltons.

1 **51.** The method of introducing a nucleic acid into a cell of claim **48**,
2 having the general structure of Formula II:



II

3
4 wherein

5 X is a member selected from the group consisting of a single bond or a
6 functional group covalently attaching said lipid to at least one ethylene oxide unit;
7 Z is a member selected from the group consisting of a single bond or a
8 functional group covalently attaching said at least one ethylene oxide unit to a cationic group;
9 and
10 n is an integer having a value of between about 6 to about 50.

1 **52.** The method of introducing a nucleic acid into a cell of claim **51**,
2 wherein

3 X is a member selected from the group consisting of a single bond,
4 phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,
5 phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate,
6 amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

1 **53.** The method of introducing a nucleic acid into a cell of claim **51**,
2 wherein

3 Z is a member selected from the group consisting of a single bond,
4 phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,
5 phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate,
6 amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

1 **54.** The method of introducing a nucleic acid into a cell of claim **51**,
2 wherein
3 A is a diacylglycerolyl moiety;
4 X is phosphoethanolamido;
5 Z is NR, wherein R is a hydrogen atom; and
6 Y is a member selected from the group consisting of about 1 to about 10 basic
7 amino acids or derivatives thereof.

1 **55.** The method of introducing a nucleic acid into a cell of claim **54**,
2 wherein
3 A is a diacylglycerolyl moiety having 2 fatty acyl chains, wherein each acyl
4 chain is independently between 2 and 30 carbons in length and is either saturated or has
5 varying degrees of saturation.

1 **56.** The method of introducing a nucleic acid into a cell of claim **54**,

2 wherein

3 Y is a member selected from the group consisting of lysine, arginine,

4 asparagine, glutamine, derivatives thereof and combinations thereof.

1 **57.** The method of introducing a nucleic acid into a cell of claim **54**,

2 wherein

3 A is a diacylglycerol moiety having 2 fatty acyl chains, wherein each acyl

4 chain is a saturated C-18 carbon chain; and

5 Y is a cationic group having 4 lysine residues or derivatives thereof.

1 **58.** The method of introducing a nucleic acid into a cell of claim **33**,

2 wherein said conjugated lipid that inhibits aggregation of particles is a PEG-lipid.

1 **59.** The method of introducing a nucleic acid into a cell of claim **58**,

2 wherein said PEG-lipid is PEG-ceramide.

1 **60.** The method of introducing a nucleic acid into a cell of claim **59**,

2 wherein the ceramide of said PEG-ceramide comprises a fatty acid group having about 8 to
3 about 20 carbon atoms.

1 **61.** The method of introducing a nucleic acid into a cell of claim **59**,

2 wherein said PEG-lipid is PEG-phosphatidylethanolamine.

1 **62.** The method of introducing a nucleic acid into a cell of claim **33**,

2 wherein said conjugated lipid that inhibits aggregation of particles is an ATTA-lipid.

1 **63.** The method of introducing a nucleic acid into a cell of claim **33**,

2 wherein said nucleic acid is selected from the group consisting of a plasmid, an antisense
3 oligonucleotide, and a ribozyme.

1 **64.** A method for inducing H_{11} phase structure in a lipid bilayer, said

2 method comprising: contacting said lipid bilayer with an endosomal membrane destabilizer,
3 thereby inducing H_{11} phase structure in a lipid bilayer.

1 **65.** The method for inducing H_{11} phase structure of claim **64**, wherein said

2 lipid bilayer comprises DOPC:DOPE:DOPS:Chol.

1 **66.** The method for inducing H_{II} phase structure of claim **64**, wherein said
2 endosomal membrane destabilizer is Ca⁺⁺ ion.

1 **67.** The method for inducing H_{II} phase structure of claim **66**, wherein Ca⁺⁺
2 ion acts in concert with low levels of the cationic lipid to trigger H_{II} phase formation.

1 **68.** Use of nucleic acid-lipid particle composition for introducing a nucleic
2 acid into a cell, said particle comprising: a cationic lipid, a conjugated lipid that inhibits
3 aggregation of particles, a nucleic acid and an endosomal membrane destabilizer.